MAJOR ARTICLE

Methicillin-Resistant Coagulase-Negative Staphylococci in the Community: High Homology of SCCmec IVa between *Staphylococcus epidermidis* and Major Clones of Methicillin-Resistant *Staphylococcus aureus*

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Background. Data on community spread of methicillin-resistant coagulase-negative staphylococci (MR-CoNS) are scarce. We assessed their potential role as a reservoir of staphylococcal cassette chromosome mec (SCCmec) IVa, the leading SCCmec subtype in community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA).

Methods. Nasal carriage of MR-CoNS was prospectively investigated in 291 adults at hospital admission. MR-CoNS were characterized by SCCmec typing, long-range polymerase chain reaction (PCR) for SCCmec IV, and multiple-locus variable-number tandem repeat analysis (MLVA) for *Staphylococcus epidermidis* (MRSE) strains. Three SCCmec IVa elements were fully sequenced.

Results. The carriage rate of MR-CoNS was 19.2% (25.9% and 16.5% in patients with and patients without previous exposure to the health care system, respectively; $P = .09$). MR-CoNS strains ($n = 83$, including 58 MRSE strains with highly heterogeneous MLVA patterns) carried SCCmec type IVa ($n = 9$, all MRSE), other SCCmec IV subtypes ($n = 9$, including 7 MRSE), other SCCmec types ($n = 15$), and nontypeable SCCmec ($n = 50$). Long-range PCR indicated structural homology between SCCmec IV in MRSE and that in MRSA. Complete sequences of SCCmec IVa from 3 MRSE strains were highly homologous to those available for CA-MRSA, including major clones USA300 and USA400.

Conclusions. MR-CoNS are probably disseminated in the community, notably in subjects without previous exposure to the health care system. MRSE, the most prevalent species, may act as a reservoir of SCCmec IVa for CA-MRSA.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been almost exclusively a health care–associated pathogen until the nineties. Community-acquired strains (CA-MRSA) have now emerged worldwide and are increasingly reported in a wide spectrum of diseases, ranging from moderate skin infections to severe necrotizing pneumonia, most often in patients with no comorbidities or risk factors [1, 2]. A few CA-MRSA clones appear highly successful in terms of geographic dissemination, for example, sequence type (ST) 80 from Europe, USA300 (ST8) and USA400 (ST1) from North America, and ST30 from Oceania, with evidence of overseas spread and in-hospital diffusion [3–5]. New CA-MRSA strains are frequently described and may emerge continuously [4, 6–10].

The acquisition of methicillin resistance in staphy-

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lococci results from the recombinase-mediated insertion of staphylococcal cassette chromosome mec (SCCmec), the mobile genetic element carrying *mecA*, at the 3' end of a chromosomal open reading frame designated as *orfX*. Eight major SCCmec types (I-VIII) are described in MRSA, differing in size and in the allotypic combination of the mec (A, B, C) and the recombinase-encoding ccr (types 1–4, ie, *ccrAB1* to *ccrAB4*, and type 5, ie, *ccrC*) gene complexes [9–14]. Major CA-MRSA clones (including USA300, USA400, and ST80) harbor SCCmec IVa, a subtype that is also currently diffusing among health care–associated MRSA (HCA-MRSA) strains [15–19]. SCCmec displays more polymorphous structure in methicillin-resistant coagulase-negative staphylococci (MR-CoNS), with frequent ccr-mec combinations not described in MRSA, and multiple and/or untypeable ccr allotypes [20–22]. Non-*mecA* SCC elements have even been reported in *Staphylococcus haemolyticus* and *Staphylococcus epidermidis*, possibly associated with arginine catabolic mobile elements (ACME) in the latter species [15, 23, 24]. Interestingly, recent data from Japan show that SCCmec IVa also predominates among community-acquired methicillin-resistant *S. epidermidis* (CA-MRSE) [25].

Several reports involving health care–associated strains suggest that SCCmec transfer from MR-CoNS to methicillin-susceptible *S. aureus* (MSSA) may occur, although its mechanism remains unknown [9, 22, 26–29]. MR-CoNS may thus act as a source of SCCmec for MRSA. The frequency of methicillin resistance in health care–associated CoNS is currently >60% [30]. On the other hand, little is known about the prevalence of methicillin resistance, the SCCmec diversity, and the reservoir of SCCmec IVa among carriage strains of CoNS, notably CA-MRSE, in western populations. Furthermore, no complete sequence of SCCmec IVa from CA-MRSE has been published so far, and homology with that carried by CA-MRSA could not be estimated. In this work, nasal carriage of MR-CoNS was prospectively studied in French adults at hospital admission. SCCmec IVa was found to be disseminated in MRSE strains, including from patients not previously exposed to the health care system, and was highly homologous to that sequenced in CA-MRSA.

STUDY POPULATION AND METHODS

Study population. All patients admitted to the orthopedic surgery ward of the Bichat–Claude Bernard teaching hospital (Paris, France) between 15 June and 16 September 2005 were prospectively enrolled and classified by means of a standardized questionnaire as previously exposed to the health care system (EHCS) or unexposed (non-EHCS) (Table 1). Patients were informed that these data may be analyzed for scientific purposes.

Isolation of staphylococci isolates from nasal swab samples. Within 6 h after ward admission, samples were obtained from both nares with a single dry-cotton swab (Copan), and directly inoculated onto Chapman (Oxoid) and MRSA Select (Bio-Rad) agar. CoNS and *S. aureus* were identified by conventional methods after incubation for 48 h at 37°C. For each subject, 2 MR-CoNS isolates were randomly collected from the MRSA Select agar, and 1 *S. aureus* strain, if present, was collected from the Chapman agar. Carriage of MS-CoNS was not investigated. The 2 MR-CoNS isolates from each patient were further considered duplicates of a single strain if they belonged to the same species and displayed identical antibiotic resistance, SCCmec types, and multiple-locus variable-number tandem repeat analysis (MLVA) patterns for *S. epidermidis* isolates (see "Molecular characterization of MR-CoNS isolates," below). Susceptibility to antibiotics (Table 2) was determined by disk diffusion (Bio-Rad) according to French Society for Microbiology guidelines [31].

Molecular characterization of MR-CoNS isolates. DNA was extracted with a MagNA Pure LC instrument (Roche). *mecA* carriage was first confirmed by multiplex real-time polymerase chain reaction (PCR) analysis [32]. Isolates were identified by (1) comparison of an internal 1350-bp fragment of the 16S ribosomal RNA gene with sequences available in the National Center for Biotechnology Information databases, using the BLAST (Basic Local Alignment Search Tool) algorithm (http://www.ncbi.nlm.nih.gov/blast) [33]; and (2) mass spectrometry (MALDI-TOF MS microflex LT, Bruker). MRSE isolates were typed by MLVA, a method that assesses the length polymorphism of 5 chromosomal variable-number tandem repeats designated as Se1–5 [34]. The diversity of MLVA patterns was analyzed with BioNumerics software (version 6.0; Applied Maths).

SCCmec was typed as described by Kondo et al [35] and designated as type I/1B (ie, *ccrAB1*–class B mec), II/2A, III/3A, IV/2B, V/5C2, VI/4B, or VIII/4A, according to the current nomenclature used for MRSA [11]. MRSA strains COL/SCCmec I, BK2464/II, ANS46c/III, USA300-FPR3757/IV(a), WCH100/V, and HDE288/VI were used as references. SCCmec IV was subtyped as IVa, IVb, IVc, IVd, and nonsubtypeable (IVnst) by multiplex PCR [35]. Nontypeable SCCmec was defined by the absence of typeable ccr allotypes or an undescribed ccr-mec combination.

Structural analysis of SCCmec IV by long-range PCR. Type IV SCCmec was compared by long-range PCR (LR-PCR) with that described in MRSA in terms of orientation and sizes of the junkyard regions J1 (from *ccrB2* toward the 3' right-flanking chromosomal region), J2 (between *mecA* and *ccrA2*), and J3 (between *orfX* and *mecA*). Primers and reference SCCmec sequences are listed in Table 3. All LR-PCR were performed by using the GeneAmp XL PCR Kit (Applied Biosystems), with an initial denaturation step (94°C for 4 min), 10 cycles of denaturation (94°C for 15 s), annealing (55°C for 30 s), and extension (68°C for 7 min), followed by 25 cycles of denatur-

Table 1. Nasal Carriage of Methicillin-Resistant Coagulase-Negative Staphylococci (MR-CoNS) and *Staphylococcus aureus* in 291 Adults at Hospital Admission

Patient characteristic	All patients	Health care system		*P*
		Exposed	Unexposed	
All patients	291	85 (29.2)	206 (70.8)	
Age, median years (range)	46 (15–103)	58 (15–103)	42 (16–92)	<.001
Female sex	116 (39.9)	43 (50.6)	73 (35.4)	.02
Exposure categories				
Hospitalization during previous year	…	72 (84.7)	…	
Life in a nursing home	…	6 (7.1)	…	
Health care worker	…	4 (4.7)	…	
Home nursing care	…	2 (2.4)	…	
Long-term hemodialysis	…	1 (1.2)	…	
Origin[a]				<.001
Emergency department	166 (57.0)	34 (40.0)	132 (64.1)	
Home	125 (43.0)	51 (60.0)	74 (35.9)	
Reason for hospitalization[a]				<.001
Urgent surgery	171 (58.8)	35 (41.2)	136 (66.0)	
Trauma with bone fracture	95 (32.6)	25 (29.3)	70 (34.0)	
Trauma without bone fracture[b]	64 (22.0)	7 (8.2)	57 (27.7)	
Nontraumatic SSTI	8 (2.7)	1 (1.2)	7 (3.4)	
Miscellaneous	4 (1.4)	2 (2.4)	2 (1.0)	
Scheduled hospitalization	120 (41.2)	50 (58.9)	70 (34.0)	
Primary surgical intervention	95 (32.7)	30 (35.3)	65 (31.6)	
Surgical reintervention	14 (4.8)	14 (16.5)	0 (0)	
Miscellaneous	11 (3.8)	6 (7.1)	5 (2.4)	
Nasal colonization by MR-CoNS, overall	56 (19.2)	22 (25.9)	34 (16.5)	.09
Nasal colonization by MRSE-SCCmec type IV	15 (5.2)	6 (7.1)	9 (4.4)	.51
Nasal colonization by *S. aureus*, overall	48 (16.5)	14 (16.5)	34 (16.5)	.87
MSSA	42 (14.4)	9 (10.6)	33 (16.0)	.3
MRSA	6 (2.1)	5 (5.9)	1 (0.5)	.01
Cocolonization by MSSA and MRSE-SCCmec type IVa	1 (0.3)	0	1 (0.5)	

NOTE. Data are no. (%) of patients, unless otherwise indicated. MRSA, methicillin-resistant *S. aureus*; MRSE–SCCmec type IV, methicillin-resistant *Staphylococcus epidermidis* carrying staphylococcal cassette chromosome mec type IV; MSSA, methicillin-susceptible *S. aureus*; SSTI, skin and soft-tissue infection.

[a] Five patients requiring urgent surgery were directly hospitalized on their general practitioner's request, without visiting the emergency room.

[b] Including deep wounds, severe sprains, and joint dislocation.

ation (94°C for 15 s), annealing (55°C for 30 s), and extension (68°C for 7–10 min, with a 7-s increment per cycle), and a final extension step (68°C for 10 min). Amplicons were analyzed after migration in 1% agar in TAE buffer 0.5×, using SYBR Safe gel stain (Invitrogen) as a double-strand DNA marker, and 1Kb Plus DNA Size Ladder (Invitrogen).

Complete sequencing of 3 SCCmec IVa. SCCmec IVa and surrounding chromosomal regions were sequenced in 3 randomly selected MRSE strains from contiguous fragments corresponding to (1) the 3 LR-PCR amplicons described above and overlapping the 3 junkyard regions; (2) the internal region of *mecA*, sequenced after conventional PCR; and (3) an additional LR-PCR amplicon for the junction between J1 and SE0130, the chromosomal coding sequence (CDS) located immediately downstream of *orfX* in *S. epidermidis* [24] (Table 3). LR-PCR amplicons were sequenced with a GA-II Genome Analyzer (Illumina). A total of 857996, 1162881, and 896106 of 76 bases readings were obtained for strains BCB-F1, BCB-F57, and BCB-F63, respectively (see Results). Readings were assembled by using a combination of de novo and comparative assembling approaches.

The reference sequence (indicated as strain/GenBank accession number/5′–3′ nucleotides position) used for comparative assembly was USA300-FPR3757/CP000255/34189–56277. The software applications used were Edena, version 2.1.1 [36]; MAQ, version 0.7.1 [37]; and Minimus2, which is part of the AMOS package, version 2.0.8 [38]. Vector NTI software (Invitrogen) was used for alignment and comparison with reference sequences of *orfX* (MRSE RP62A/CP000029/2584176–2584655, methicillin-susceptible *S. epidermidis* [MSSE] Amer-

Table 2. Antibiotic Resistance Patterns of 83 Carriage Strains of Methicillin-Resistant Coagulase-Negative Staphylococci (MR-CoNS)

Antibiotic resistance	MR-CoNS strains, no. (%)			
	Total (n = 83)	EHCS subjects (n = 32)	Non-EHCS subjects (n = 51)	P
Oxacillin	83 (100)	32 (100)	51 (100)	
Kanamycin	47 (56.6)	25 (78.1)	22 (43.1)	.004
Tobramycin	45 (54.2)	24 (75.0)	21 (41.2)	.005
Gentamicin	28 (33.7)	11 (34.3)	17 (33.3)	.88
Tetracycline	21 (25.3)	7 (21.9)	14 (27.4)	.76
Rifampicin	8 (9.6)	5 (15.6)	3 (5.9)	.28
Fosfomycin	24 (28.9)	9 (28.1)	15 (29.4)	.90
Fusidic acid	33 (39.8)	13 (40.6)	20 (39.2)	.92
Erythromycin	63 (75.9)	28 (87.5)	35 (68.6)	.09
Lincomycin	18 (21.7)	10 (31.2)	8 (15.7)	.16
Pristinamycin	1 (1.2)	1 (3.1)	0	…
Ofloxacin	28 (33.7)	16 (50.0)	12 (23.5)	.02
Cotrimoxazole	20 (24.1)	9 (28.1)	11 (21.6)	.68
Vancomycin	0	0	0	…

NOTE. Antibiotic susceptibility/resistance was determined by the disk diffusion method, according to the French Society for Microbiology guidelines [31]. EHCS, exposed to health care system.

ican Type Culture Collection [ATCC] 12228/AE015929/32031–32510, CA-MRSA USA300-FPR3757CP000255/33711–34191, CA-MRSA USA400-MW2/BA000033/33688–34168), SCCmec IVa (CA-MRSA USA300-FPR3757/CP000255/34189–56277, CA-MRSA USA400-MW2/BA000033/34145–57500, CA-MRSA CA05-JCSC1968/AB063172/976–25205), SE0130 (MRSE RP62A/CP000029/2491223–2492038, and MSSE ATCC 12228/AE015929/126025–126840) [14–16, 24, 39]. All divergences from reference sequences (ie, nucleotidic substitutions, insertions, and deletions) were controlled for with specifically designed primers.

Statistical analysis. The χ^2 test and analysis of variance were used to compare nominal and continuous variables, respectively. Differences were considered significant at $P<.05$.

RESULTS

***Carriage rates of MR-CoNS and* S. aureus.** A total of 291 consecutive patients were enrolled in the study, including 85 EHCS patients (29.2%) and 206 non-EHCS patients (70.8%) (Table 1). Compared with non-EHCS subjects, EHCS patients were older (median age, 58 vs 42 years; $P<.001$) and were more likely to have been admitted directly from home (60% vs 35.9%; $P<.001$) for scheduled surgery (58.9% vs 34%; $P<.001$). The overall prevalence of MR-CoNS carriage was 19.2% ($n = 56$). Carriage rates in EHCS and non-EHCS subjects were 25.9% and 16.5%, respectively ($P = .09$). Forty-eight patients (16.5%) carried *S. aureus*, including 6 with MRSA, with no MRSA or MR-CoNS cocolonization.

MR-CoNS strains. Isolates ($n = 112$) were duplicates of a single strain in 29 patients and represented 2 distinct strains in the other 27 patients, including 16 with strains from different species. The remaining 83 nonduplicate MR-CoNS strains included in the analysis were identified as *S. epidermidis* (58 strains [69.9%]), *Staphylococcus hominis* (11 strains [13.3%]), *S. haemolyticus* (10 strains [12.0%]), *Staphylococcus pettenkoferi* (3 strains [3.6%]) (a recently described species [40]), and *Staphylococcus cohnii* (1 strain [1.2%]). Only resistances to kanamycin, tobramycin, and ofloxacin were significantly more frequent in MR-CoNS strains from EHCS than from non-EHCS subjects (Table 2).

Type IV was the most frequent SCCmec variant (21.7%, see below) (Table 4). SCCmec II and V were identified in 3 *S. epidermidis* and 5 *S. haemolyticus* strains, respectively. Seven strains (including 4 MRSE) carried a 4A combination, designated in MRSA as SCCmec VIII [9, 11]. Fifty strains (60.2%) harbored nontypeable SCCmec, with 19 distinct patterns, including double and triple ccr allotypes for 23 (27.7%) and 3 (3.6%) strains, respectively. Distribution of SCCmec types did not differ significantly between MR-CoNS from EHCS and non-EHCS patients.

Nasal colonization by MR-CoNS strains carrying SCCmec type IV. Seventeen patients (5.8%) were colonized by MR-CoNS strains ($n = 18$) carrying SCCmec type IV (9.4% in EHCS vs 4.4% in non-EHCS subjects; not significant). Sixteen strains (88.8%) were identified as *S. epidermidis*, with a corresponding carriage rate for MRSE-SCCmec IV of 5.2% (7.1% in EHCS vs 4.4% in non-EHCS patients; not significant), and 2 were identified as *S. pettenkoferi*. One subject carried 2 distinct strains of MRSE-SCCmec IV, and 6 were cocolonized by 1 strain with SCCmec IV (4 *S. epidermidis* and 2 *S. pettenkoferi*) plus another strain harboring a non–type IV SCCmec. One non-EHCS subject carried MSSA as well as CA-MRSE with SCCmec IVa.

MLVA typing of MRSE strains. Fifty-five (94.8%) of the 58 distinct MRSE strains were typeable by MLVA, with ⩾3 of the 5 targeted loci not amplifiable in the remaining 3 strains. These 55 strains showed heterogeneous MLVA profiles, regardless of their origin (EHCS or non-EHCS patients) or their SCCmec types (notably type IV), with no patent clonality (Figure 1).

Characterization of SCCmec type IV. SCCmec IVa was the most frequent subtype ($n = 9$) among MRSE-SCCmec IV, followed by IVnst ($n = 6$) and IVb ($n = 1$) (Table 4). *S. pettenkoferi* strains carried subtypes IVc and IVd.

LR-PCR indicated a marked structural homology of SCCmec IV from MRSE with that described in MRSA. Insertion of SCCmec at the 3′-end of *orfX* was confirmed in all strains, with

Table 3. Primers Used for Long-Range Polymerase Chain Reaction Amplification of Staphylococcal Cassette Chromosome mec (SCCmec) Type IV and Additional Primers Used for Complete Sequencing of SCCmec Subtype IVa

Primers and corresponding regions	Constructed on	5′–3′ Sequence	Reference SCCmec		
			Strain[a]	Start position, nucleotide	Length, bp
From *orfX* to mec (overlapping J3)					
orfX-U2R[b]	*orfX*	GTCAAAGAAAAAGAAGGCCAACG	FPR3757 (IVa) JCSC1978 (IVb) MR108 (IVc)	33877 21516 30864	6213 (IVa) 6830 (IVb) 9481 (IVc)
mA1[c]	*mecA*	TGCTATCCACCCTCAAACAGG	FPR3757 (IVa) JCSC1978 (IVb) MR108 (IVc)	40090 14686 21383	6213 (IVa) 6830 (IVb) 9481 (IVc)
From mec to *ccrA2* (overlapping J2)[c]					
mA7	*mecA*	ATATACCAAACCCGACAACTACA	FPR3757 (IVa) JCSC1978 (IVb) MR108 (IVc)	41073 13703 20400	6904 (IVa) 6904 (IVb) 6905 (IVc)
α2	*ccrA2*	TAAAGGCATCAATGCACAAACACT	FPR3757 (IVa) JCSC1978 (IVb) MR108 (IVc)	47977 6799 13495	6904 (IVa) 6904 (IVb) 6905 (IVc)
From *ccrB2* to 3″-SCCmec (overlapping J1)[c]					
Bc (all subtypes)	*ccrB2*	ATTGCCTTGATAATAGCCITCT	FPR3757 (IVa) JCSC1978 (IVb) MR108 (IVc) JCSC4469 (IVd)	47041 7735 14431 11066	7122 (IVa) 5278 (IVb) 6171 (IVc) 8676 (IVd)
4a1 (IVa)	J1 IVa	TTTGAATGCCCTCCATGAATAAAAT	FPR3757 (IVa)	54163	7122 (IVa)
4b3 (IVb)	J1 IVb	AACCAACAGTGGTTACAGCTT	JCSC1978 (IVb)	2457	5278 (IVb)
4c4 (IVc)	J1 IVc	AGGAAATCGATGTCATTATAA	MR108 (IVc)	8260	6171 (IVc)
4d4 (IVd)	J1 IVd	AATTCACCCGTACCTGAGAA	JCSC4469 (IVd)	2390	8676 (IVd)
Internal region of *mecA*[b,d]					
mecED2	*mecA*	ACGTGGAGACGAGCACTAATAACCA	FPR3757	41374	1516
mecER	*mecA*	TTTTGCCAACCTTTACCATCGATT	FPR3757	39833	1516
mecID1	*mecA*	AGACCGAAACAATGTGGAATTGG	FPR3757	40669	…
mecIR1	*mecA*	CCAATTCCACATTGTTTCGGTCT	FPR3757	40647	…
From J1 to the 3′ chromosomal flanking region[d]					
4a3[c]	J1 IVa	AGAAAAGATAGAAGTTCGAAAGA	FPR3757	53706	…
SE130–360[b]	SE0130	GATTGTTTTATTTAGCGGCGAGC	ATCC 12228	126481	…

[a] The GenBank accession numbers of methicillin-resistant *Staphylococcus aureus* (MRSA) reference strains are CP000255 (USA300-FPR3757), AB063173 (JCSC1978), AB096217 (MR108), and AB097677 (JCSC4469). Only the junkyard region J1 of SCCmec IVd was sequenced in MRSA strain JCSC4469. The GenBank accession number of methicillin-susceptible *Staphylococcus epidermidis* strain American Type Culture Collection 12228 is AE015929.

[b] Primers designed for the purpose of this study.

[c] Primers from reference 35.

[d] Additional primers for complete sequencing of SCCmec IVa. medID1 and medIR1 primers were only used for *mecA* sequencing. The length from J1 to the 3′ chromosonal flanking region is not available.

~6.5-kb amplicons reflecting a J3 region similar in orientation and size to those sequenced in MRSA USA300-FPR3757 (IVa) and JCSC1978 (IVb) (6213 and 6830 bp, respectively). A ~7-kb J2 amplicon was obtained in each strain, with a corresponding mec-ccr distance of 6904 bp in reference strains of MRSA (Table 3). The 9 strains with SCCmec IVa harbored an upstream J1 region of ~7 kb, as in USA300-FPR3757 (7122 bp). This region was ~5.5 kb long in the strain with SCCmec IVb, similar to JCSC1978 (5278 bp). Only J2 was amplified in *S. pettenkoferi* strains (amplicons ~7 kb).

Sequencing of SCCmec IVa. Three MRSE strains were randomly selected for SCCmec IVa sequencing (Table 5 and Figure 2). Strain BCB-F1 was isolated in a 78-year-old woman admitted for hip fracture (EHCS; hospitalization within the previous year for acute renal failure), BCB-F57 in a 55-year-old woman hospitalized for scheduled hand surgery (non-EHCS; no relevant medical history), and BCB-F63 in a 83-year-old man with shoulder dislocation (EHCS; resident of a rest home, no previous hospitalization). Overall sequence identity with SCCmec IVa from CA-MRSA strain USA300-FPR3757 was

Table 4. ccr-mec Allotype Combinations in 83 Carriage Strains of Methicillin-Resistant Coagulase-Negative Staphylococci and Staphylococcal Cassette Chromosome mec (SCCmec) Assignment According to Current Classification Used for Methicillin-Resistant *Staphylococcus aureus*

ccr-mec Combination; SCCmec type assignment[a]	No. (%) of strains			*Staphylococcus* species (no. of isolates)
	Overall	Non-EHCS patients	EHCS patients	
2A: type II	3 (3.6)	2 (3.9)	1 (3.1)	*S. epidermidis* (3)
2B: type IV	18 (21.7)	9 (17.6)	9 (28.1)	
Subtype IVa	9	3	6	*S. epidermidis* (9)
Subtype IVb	1	1	0	*S. epidermidis* (1)
Subtype IVc	1	0	1	*S. pettenkoferi* (1)
Subtype IVd	1	0	1	*S. pettenkoferi* (1)
IVnst	6	5	1	*S. epidermidis* (6)
5 C2; type V	5 (6.0)	3 (5.9)	2 (6.2)	*S. haemolyticus* (5)
4 A; type VIII	7 (8.4)	4 (7.8)	3 (9.4)	*S. epidermidis* (4), *S. hominis* (3)
Nontypeable	50 (60.2)	33 (64.7)	17 (53.1)	
NT A	7	5	2	*S. epidermidis* (5), *S. hominis* (2)
NT B	4	2	2	*S. epidermidis* (1), *S. haemolyticus* (3)
NT C2	1	1	0	*S. epidermidis* (1)
1 A	5	4	1	*S. hominis* (4*), S. pettenkoferi* (1)
2 C2	4	1	3	*S. epidermidis* (4)
2 NT	1	0	1	*S. epidermidis* (1)
4 C2	1	1	0	*S. epidermidis* (1)
5 A	1	1	0	*S. cohnhii* (1)
1–2 B	2	1	1	*S. epidermidis* (2)
1–2 C2	1	0	1	*S. hominis* (1)
1–4 A	2	2	0	*S. epidermidis* (1), *S. hominis* (1)
1–5 C2	1	1	0	*S. haemolyticus* (1)
2–4 A	2	0	2	*S. epidermidis* (2)
2–4 B	4	4	0	*S. epidermidis* (4)
2–5 A	3	1	2	*S. epidermidis* (3)
2–5 B	2	0	2	*S. epidermidis* (2)
2–5 C2	5	5	0	*S. epidermidis* (4), *S. haemolyticus* (1)
4–5 C2	1	1	0	*S. epidermidis* (1)
2–4–5 A	3	3	0	*S. epidermidis* (3)
Total	83	51	32	

NOTE. EHCS, exposed to health care system.

[a] ccr allotypes are classified as types 1–4 (*ccrAB1* to *ccrAB4*) and 5 (*ccrC*); *ccrAB2*, *ccrC*, and *ccrAB1* were more frequent in *S. epidermidis* (75% vs 16% of strains from other species; $P<.001$), *S. haemolyticus* (70.0% vs 20.0%; $P=.003$), and *S. hominis* (54.5% vs 6.8%; $P<.001$) strains, respectively. SCCmec types are as defined in reference 11. IVnst, nonsubtypeable SCCmec type IV; NT, nontypeable mec-ccr allotype.

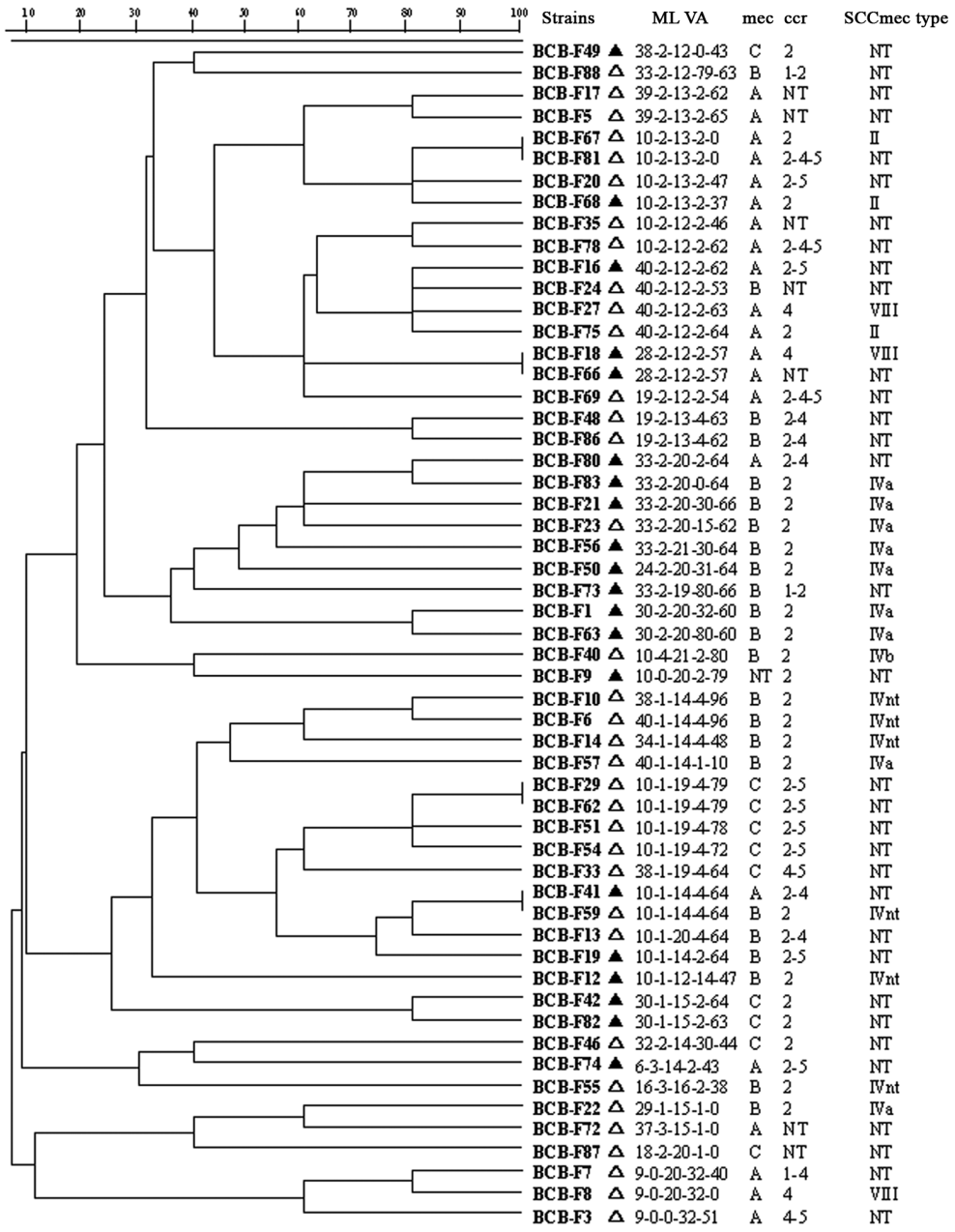

Figure 1. Genetic relationship of multiple-locus variable-number tandem repeat analysis (MLVA) patterns for methicillin-resistant *Staphylococcus epidermidis* (MRSE) strains, as implemented by the BioNumerics software (version 6.0; Applied Maths). SCCmec, staphylococcal cassette chromosome mec, defined by the ccr-mec allotype combination according to the current classification used in methicillin-resistant *Staphylococcus aureus* [11]; NT, nontypeable ccr-mec allotypes or SCCmec types. Strains are MRSE strains as isolated from subjects with (*solid triangles*) or without (*open triangles*) previous exposure to the health care system, MLVA patterns are defined as the number of tandem repeats for each of the 5 Se loci (Se1-Se2-Se3-Se4-Se5) [34], and ccr allotypes are classified as types 1–4 (*ccrAB1–ccrAB4*) and 5 (*ccrC*).

99.99% for MRSE strains BCB-57 and BCB-F63 and 96.63% for MRSE strain BCB-F1.

BCB-F1 carried an SCCmec IVa sequence ~22.7 kb long sharing 100% nucleotidic homology for J1 and J2 regions to its counterparts in USA300-FPR3757, for CDSs as for intergenic sequences. These 2 cassettes differed only in 2 intergenic sequences of their J3 regions by (1) the insertion in BCB-F1 of 2 fragments of 578 bp (at nucleotide 1656) and 39 bp (at nucleotide 3716) that were 100% homologous to the corresponding region of SCCmec IVa in CA-MRSA strain USA400-MW2 (GenBank BA000033, nucleotides 35795–36372 and nucleotides 37854–37893, respectively) and (2) the deletion in

Table 5. CDS Lists of Staphylococcal Cassette Chromosome mec (SCCmec) Type IVa in 3 Strains of Methicillin-Resistant *Staphylococcus epidermidis*

	Reference				Strain BCB-F1			Strain BCB-F57			Strain BCB-F63		
Region	CDS (strain[a])	Length, bp	Protein, amino acids	Function	CDS	Start, nucleotide	Sequence identity, %	CDS	Start, nucleotides	Sequence identity, %	CDS	Start, nucleotide	Sequence identity, %
Chromosome[b]	*orfX* –partial (ATCC 12228)	30	NA	…	F1–01	1	100	F57–01	1	95	F63–01	1	100
SCCmec J3	SAUSA300_027 (FPR3757)	1257	418	CHP	F1–02	352	100	F57–02	351	100	F63–02	352	100
	SAUSA300_028 (FPR3757)	675	224	Transposase[c]	F1–03	2294	100	F57–03	1715	99	F63–03	1716	100
	SAUSA300_029 (FPR3757)	168	55	CHP	F1–04	3226	100	F57–04	2647	100	F63–04	2648	100
	SAUSA300_030 (FPR3757)	744	247	GDP[c]	F1–05	4150	100	F57–05	3651	100	F63–05	3652	100
	SAUSA300_031 (FPR3757)	429	142	CHP	F1–06	4990	100	F57–06	4491	100	F63–06	4492	100
mec gene complex	*mecA* (FPR3757)	2007	668	PBP2a	F1–07	5464	100	F57–07	4965	100	F63–07	4966	100
	mecR1 (FPR3757)	987	328	*mecA* regulator	F1–08	7570	100	F57–08	7071	100	F63–08	7072	100
SCCmec J2	SAUSA300_035 (FPR3757)	216	71	CHP	F1–09	8573	100	F57–09	8074	100	F63–09	8075	100
	IS1272 (FPR3757)	1512	503	Transposase	F1–10	8779	100	F57–10	8280	99	F63–10	8281	100
	SAUSA300_036 (FPR3757)	507	168	CHP	F1–11	10438	100	F57–11	9939	100	F63–11	9940	100
ccr gene complex	*ccrB2* (FPR3757)	1629	542	Recombinases	F1–12	12229	100	F57–12	11730	100	F63–12	11731	100
	ccrA2 (FPR3757)	1350	449	A2/B2	F1–13	13879	100	F57–13	13380	100	F63–13	13381	100
SCCmec J1	SAUSA300_039 (FPR3757)	1788	595	CHP	F1–14	15462	100	F57–14	14963	100	F63–14	14964	100
	SAUSA300_040 (FPR3757)	291	96	CHP	F1–15	17249	100	F57–15	16750	100	F63–15	16751	100
	SAUSA300_041 (FPR3757)	1092	363	CHP	F1–16	17636	100	F57–16	17137	100	F63–16	17138	100
	SAUSA300_042 (FPR3757)	1491	496	CHP	F1–17	19455	100	F57–17	18956	99	F63–17	18957	100
	SAUSA300_043 (FPR3757)	174	57	CHP	F1–18	21318	100	F57–18	20819	100	F63–18	20820	100
	SAUSA300_044 (FPR3757)	1005	334	CHP	F1–19	21569	99	F57–19	21069[d]	100	F63–19	21071	99
Chromosome[b]	SE0130-partial (ATCC 12228)	250	NA	…	F1–20	22670	99	…	…	…	F63–20	22172	99

NOTE. CDS, coding sequence; CHP, conserved hypothetical protein; GDP, glycerophosphoryl diester phosphodiesterase; J, junkyard region; NA, not applicable; PBP2a, penicillin-binding protein 2A.

[a] The GenBank accession numbers are AE015929 for methicillin-susceptible *S. epidermidis* strain ATCC 12228 and CP000255 for methicillin-resistant *Staphylococcus aureus* strain USA300-FPR3757.

[b] Only the 3′ end of *orfX* (total length, 480 bp) and the 5′end of SE0130 (total length, 816 bp) were sequenced in the 3 *S. epidermidis* strains.

[c] Putative function.

[d] Partial (sequence length, 322 bp).

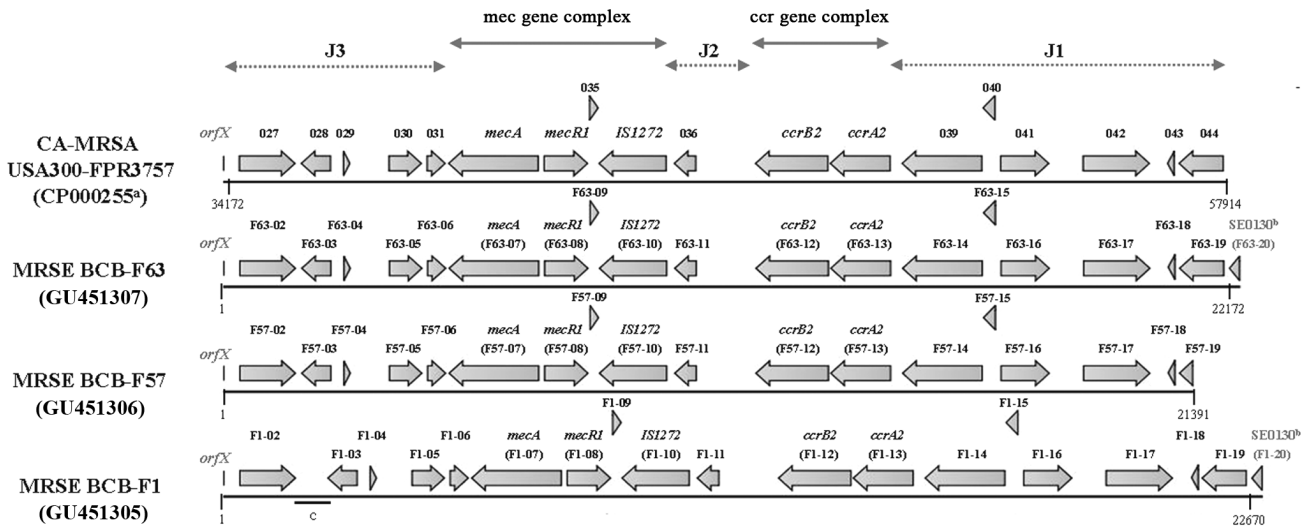

Figure 2. Structure and chromosomal coding sequence (CDS) content of staphylococcal cassette chromosome mec (SCCmec) IVa in methicillin-resistant *Staphylococcus epidermidis* (MRSE) strains BCB-F1, BCB-F57, and BCB-F63 and community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strain USA300-FPR3757. CDSs located within SCCmec are indicated in black, and chromosomal CDSs in gray; GenBank accession numbers are provided in brackets. The first 30 nucleotides in MRSE strains correspond to the 3′ end of *orfX*. All of the CDSs located on SCCmec IVa in MRSE strains displayed >99% nucleotidic homology relative to their counterparts in USA300-FPR3757. [a]Findings as annotated by Diep et al [15]. The type I arginine catabolic mobile element (ACME) located downstream of SCCmec in USA300-FPR3757 (nucleotides 57914–88899) is not represented here. [b]SE0130 is the chromosomal CDS located immediately downstream of *orfX* in methicillin-susceptible *S. epidermidis* strain American Type Culture Collection 12228 (GenBank accession no. AE015929). The junction between J1 and SE0130 was not amplifiable in the ACME-*copA*–positive strain BCB-F57 (see text for details). [c]Two fragments of 578 bp (at nucleotide 1656) and 39 bp (at nucleotide 3716) were inserted in the J3 region of SCCmec IVa in strain BCB-F1 and were 100% homologous to the corresponding region of SCCmec IVa in CA-MRSA strain USA400-MW2 (GenBank accession no. BA000033, nucleotides 35795–36372 and nucleotides 37854–37893, respectively).

BCB-F1 of 2 fragments of 79 bp (at nucleotide 3821) and 39 bp (at nucleotide 3991), with 12-point mutations between these 2 locations. The remaining intergenic sequences and all of the CDSs located on J3 in BCB-F1 displayed >99% and 100% homology, respectively, with their counterparts in USA300-FPR3757. MRSE strain BCB-F63 carried an SCCmec IVa sequence ~22.1 kb long that was 100% and >99% homologous to that sequenced in USA300-FPR3757 for CDSs and intergenic sequences, respectively.

CDSs F1–20 and F63–20 were >99% homologous to the SE0130 locus of MSSE strain ATCC 12228, which confirmed the connection between the 3′ boundaries of SCCmec and the right-flanking chromosomal regions of strains BCB-F1 and BCB-F63.

An ~21.1-kb-long sequence of SCCmec IVa was obtained in BCB-F57, with 14 and 3 CDSs displaying 100% and >99% homology with their counterparts in USA300-FPR3757, respectively. The 3′ SCCmec-chromosome junction could not be amplified by LR-PCR in this strain, which suggested the insertion in *orfX* of another mobile genetic element downstream of SCCmec IVa. Three markers of ACME, namely, *arcA*, *opp3AB,* and *copA*, were screened by PCR, using USA300-FPR3757 as a positive control [41]. Only *copA* was amplified. An additional LR-PCR assay indicated that *copA* was located ~4 kb upstream of SE0130 in BCB-F57.

These 3 SCCmec IVa sequences displayed >99.9% sequence identity with their counterparts in other reference strains of CA-MRSA (ie, USA400-MW2 and CA05-JCSC1968), except for 2 intergenic sequences of J3, as described for strain BCB-F1 (data not shown).

GenBank accession numbers. Sequences of SCCmec IVa with surrounding partial sequences of *orfX* (for the 3 strains) and SE0130 (for BCB-F1 and BCB-F63) are available at http://www.ncbi.nlm.nih.gov/GenBank under numbers GU451305 (BCB-F1), GU451306 (BCB-F57), and GU451307 (BCB-F63).

DISCUSSION

In this study, carriage of MRSE-SCCmec type IV was found to be common in patients at hospital admission, including those with no previous exposure to the health care system, with an overall colonization rate of 5.2%. LR-PCR assays confirmed that structures of these SCCmec IV were similar to those described in MRSA. Moreover, we provide complete sequences of SCCmec IVa from MRSE strains circulating in the community, with a high homology to sequences of SCCmec IVa carried by major CA-MRSA clones USA300 and USA400.

Worldwide emergence of numerous unrelated CA-MRSA clones has been one of the most alarming phenomena in terms of antibiotic resistance over the past decade. Most of these clones carry SCCmec IVa [7, 15, 16, 19]. The small size of this SCCmec variant may enhance its mobility and ease its current spread among MRSA in the community, as in the hospital [17, 18], with a possible impact of frequent vancomycin use in the latter setting [42]. Several reports suggest that new HCA-MRSA strains can arise as a result of SCCmec transfer between MR-CoNS and MSSA [22, 26–28]. It could be hypothesized that some CA-MRSA clones have emerged from a similar, community-based event. As recently reported in Japan [25], we observed that SCCmec IVa was disseminated among carriage strains of MRSE. Moreover, cocolonization by MSSA and MRSE-SCCmec IVa was found to be possible in patients at hospital admission. In this context, community diffusion and silent in-hospital influx of MRSE-SCCmec IV may reflect a widely accessible reservoir of methicillin resistance for *S. aureus* in these 2 environments.

Very few SCCmec elements have been fully sequenced in MRSE [29, 39]. One study reported >98% nucleotide identity for SCCmec IVa between MRSA strain CA05-JCSC1968 and clinical MRSE strains causing prosthetic valve endocarditis, a mainly health care–associated infection. SCCmec sequences from these MRSE strains are not available for comparison in the GenBank database [29]. In our study, sequencing of SCCmec IVa in 3 MRSE strains (including 2 from patients with no previous hospitalization) provided further arguments for SCCmec exchange between *S. aureus* and *S. epidermidis*. Strain BCB-F63 harbored SCCmec IVa with 99.99% nucleotide identity compared with the cassette sequenced in USA300-FPR3757 [15]. A similar observation was made in strain BCB-F1, except for the 5'-end of the J3 region, which may result from recombination with another SCCmec IVa element identical to that sequenced in USA400-MW2 [15, 16]. Finally, strain BCB-F57 carried SCCmec IVa that was 99.99% identical to its counterpart in USA300-FPR3757, with the likely insertion of another mobile genetic element between the 3′ end of SCCmec and the right-flanking chromosomal locus (SE0130).

Interestingly, this additional region contained *copA,* an ACME-associated gene located immediately upstream of the right chromosomal junction of the SCCmec IVa-ACME type I composite element in USA300-FPR3757 and of ACME type II in MSSE strain ATCC 12228 [15, 24]. However, BCB-F57 did not carry the arc operon integrated in ACME types I and II nor the *opp3* operon, which was described only in ACME type I. Spontaneous, subtotal deletion of an ACME located downstream of SCCmec IVa might have occurred in BCB-F57 or its precursor. A degree of homologous recombination between distinct SCC elements is another eventuality, as recently suggested for SCCmec VIII in MRSA strain C10682 [9]. Nevertheless, these data constitute one more line of evidence supporting the role of MRSE as a pool of SCCmec IVa for *S. aureus.*

As we studied MRSE strains probably circulating in French community settings and hospitals, comparison with complete sequences of SCCmec IVa from the leading European CA-MRSA clone ST80 would have been of major interest to further assess this hypothesis. Unfortunately, these sequences are not yet deposited in GenBank. Only a partial, conserved *ccrA2* sequence from ST80 is currently available (GenBank accession number AY669512) [43], and it is 100% homologous to those obtained in our 3 strains.

The overall biodiversity of MR-CoNS colonizing community subjects has been scarcely assessed [21, 25, 44]. We confirm here the extreme structural heterogeneity of SCCmec elements among CA-MR-CoNS strains, with two-thirds of them carrying untypeable ccr-mec combinations. No significant difference was found in this respect with strains from EHCS subjects. This high polymorphism in community-acquired strains, notably in CA-MRSE, suggests that rearrangements and horizontal transfers of this resistance island frequently occur despite a relatively low antibiotic exposure. Data from MLVA support this evidence, as nearly all MRSE strains harbored a distinct genetic background, whether they carried a type IV or another SCCmec element. Similar findings were recently reported in CA-MRSE strains from Japan [25]. This observation contrasts with results of SCCmec typing in *S. aureus,* with most CA-MRSA strains carrying well-defined SCCmec patterns, such as types IV and V. Genomic plasticity in CoNS species, as suggested for *S. epidermidis* [24, 39] and *S. haemolyticus* [23], may ease sequential insertions of foreign genetic elements in *orfX* and contribute to the frequent generation of new SCC elements.

Regardless of SCCmec typing, we found that almost 20% of patients were MR-CoNS carriers at hospital admission, with a trend toward a higher prevalence in EHCS subjects. Hospitalization in the previous year, long-term hemodialysis, nursing care at home, and living in a rest home increase the risk of HCA-MRSA nasal carriage [45]. Our data suggest that similar risk factors exist for MR-CoNS colonization, in agreement with the demonstrated impact of antibiotic pressure and cross-transmission on this carriage in hospitalized patients [46]. More strikingly, 16.5% of non-EHCS subjects carried MR-CoNS, with high rates of non–β-lactam coresistances. The diffusion of MR-CoNS in individuals with no underlying risk factor has been recently reported in non-European populations [21, 25, 44]. This spread may elicit additional concerns, given that CoNS are increasingly reported in community-acquired diseases, such as native-valve endocarditis and late-onset infections of prosthetic heart valves, pacemakers, and orthopedic prostheses [47–49].

Our work has some limitations that should be mentioned. First, patients were classified as EHCS or non-EHCS on the

basis of a questionnaire including items on hospitalization within the previous year, home nursing care, long-term hemodialysis, life in a nursing home, and health care jobs. We cannot exclude the possibility that some non-EHCS patients had experienced earlier hospitalization (>1 year) or emergency department visits without hospital admission, and the impact of such exposure on MR-CoNS carriage is currently unknown. Next, nasal samples were obtained in the orthopedic ward, not in the emergency department. However, isolation at ward admission of a MR-CoNS strain acquired during emergency department transit is unlikely, because emergency department stays in our hospital are usually <6 h as a result of prompt ward transfer policy. Finally, reliable retrospective data on antibiotic use during the months before inclusion were unavailable, notably for non-EHCS subjects. Consequently, we could not confirm that antibiotic exposure increases the MR-CoNS carriage rate in these patients, as suggested elsewhere [50].

In conclusion, this study provides new data concerning the role of MR-CoNS as a wide and evolutionary pool of SCCmec in the community, as in hospitalized patients. Most notably, our results argue for exchanges of SCCmec IVa between *S. epidermidis* and *S. aureus*, a worrisome report for western European countries, where incidence of CA-MRSA remains still relatively low [51].

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